

# First Structure Solved by Anomalous Methods at the Canadian Light Source

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## Introduction

Inositol dehydrogenase (IDH) from *Bacillus subtilis* is a NAD<sup>+</sup>-dependent enzyme that catalyses the oxidation of the axial hydroxyl group of myo-inositol to form scyllo-inosose (Figure 1). IDH is a member of the GFOR/IDH/MocA family, a group of homologous dehydrogenases. IDH has a molecular mass of 39.17 kDa, but is found as a tetramer in solution, with an apparent

molecular mass of 160 kDa [2]. Recently, an IDH model has been generated [1] based on the crystal structure of GFOR from *Zymomonas mobilis* (PDB code 1OFG) [1,3]. Sequence alignment with IDH homologues and homology modelling of IDH allowed us to predict active site residues important for binding and catalysis. H176 and D172 are proposed to act as the catalytic diad, in which H176 is proposed to be the acid/base catalyst. Mutants of H176A and D172N showed a marked loss in activity [1]. However, knowledge of their actual role in catalysis requires determining the precise role of active site residues as provided by a high resolution crystal structure with and without substrates or inhibitors.

## Method

Details of the over-expression and crystallization conditions for native IDH and a selenomethionine-substituted variant (SeMet-IDH) were published in 2008 [4]. A two wavelength MAD dataset to 1.75 Å resolution was collected on beamline 08ID-1 at the Canadian Light Source. A clear selenium absorption edge was observed in the XAFS experiment, enabling us to determine the peak, inflection and remote wavelengths (Figure 2). The diffraction data consisted of a total of 180 images, each exposed for 1 s with 1.0° oscillation at a crystal-to-detector distance of 180 mm. The images were integrated and scaled using XDS/ XSCALE [5].

## Results and discussion

For molecular replacement, the program MRBUMP [6] with PHASER [7] from the CCP4 suite was used, with search models that included structures with 15 to 22 % sequence identity to IDH. A clear molecular replacement solution was found using a monomer of PDB entry 2GLX (NADP(H)-Dependent 1,5-Anhydro-D-fructose Reductase from *Sinorhizobium morelense*; 22% sequence identity to IDH) as the search model. However, this solution did not allow refinement of the structure.

SeMet IDH crystallizes in the same space group and cell dimensions as native IDH. We have collected the first MAD

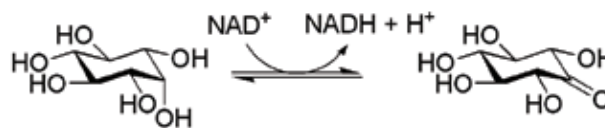


Figure 1: Reaction catalyzed by inositol dehydrogenase (IDH)

dataset ever collected at the Canadian Light Source. The crystal diffracted to 1.75 Å resolution. The statistics of data collection and phasing are summarized in Table 1. A search for Se-atom sites was carried out with SHELXD [8,9], resulting in 5 out of 6 Se sites, assuming six SeMet residues in one molecule. Phases were further improved by density-modification methods using DM (Collaborative Computational Project, Number 4, 1994). The resulting electron-density map allowed tracing of the main chain of the polypeptides.

## Conclusion

The first MAD data was collected from a SeMet IDH crystal on the CLS beamline 08ID-1, Canadian Macromolecular Crystallography Facility (CMCF1). The crystal diffracted to 1.75 Å. Good quality MAD data was obtained. Similar data was collected at the Advanced Photon Source (Argonne, Ill), with similar results [4]. This reports the first high quality MAD diffraction data acquired at the CLS.

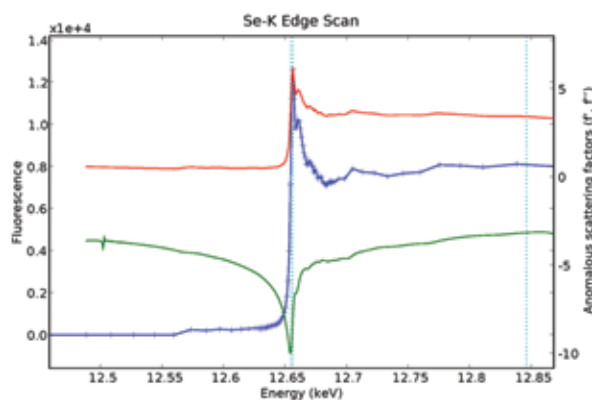


Figure 2: Scattering factors ( $f'$  and  $f''$ ) from SeMet\_IDH crystals collected at beamline 08ID-1. The energies indicated correspond to the beamline calibration.

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Values in parentheses are for the highest resolution shell			
	Peak	Inflection	Peak
Beam line	CLS	CLS	APS
Wavelength (Å)	0.9797	0.9798	0.9793
Space group	I222		I222
Unit cell parameters (Å)	a=52.5, b=120.4, c=129.2		a=51.2, b=122.4, c=129.4
Resolution (Å)	29.3-1.75 (1.81-1.75)	29.3-1.75 (1.81-1.75)	50-2.04 (2.12-2.04)
Observed reflections	275659 (25099)	274938 (24974)	194112 (25789)
Unique reflections	41186 (3964)	41116 (3952)	48289 (3687)
Completeness (%)	98.7 (96.3)	98.7 (96.2)	96.4 (73.1)
Multiplicity	6.7 (6.3)	6.7 (6.3)	4.0 (3.1)
I/σ(I)	10.5 (3.2)	10.3 (3.5)	27.3 (4.3)
Rmerge †	7.8 (45.6)	7.8 (42.1)	4.6 (14.5)
Phasing statistics			
Se sites (found/all)	5/6		5/6
FOM after SHELXD	0.35		0.34
† Rmerge = $\sum  I_i - \langle I_i \rangle  / \sum \langle I_i \rangle$ where $I_i$ is the observed intensity and $\langle I_i \rangle$ is the average intensity over symmetry-equivalent measurements			

**Table 1:** Data-collection statistics from CLS 08ID-1 and APS 24 ID

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